New Steroidal Saponins from Rhizomes of *Costus spiralis*

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Z. Naturforsch. 59c, 81–85 (2004); received May 21, 2003

Two new steroidal saponins were isolated from the rhizomes of *Costus spiralis* Rosc. Their structures were established as (3β,25R)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl O-β-D-apio-β-D-furanosyl-(1→2)-O-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (1) and (3β,25R)-26-(β-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl O-β-D-apio-β-D-furanosyl-(1→4)-O-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside (2). Their structural identifications were performed using detailed analyses of 1H and 13C NMR spectra including 2D NMR spectroscopic techniques (DEPT, COSY, HETCOR and COLOC) and chemical conversions. The steroidal saponins were evaluated for anti-inflammatory activity.

Key words: *Costus spiralis*, Steroidal Saponins, Anti-inflammatory Activity

Introduction

*Costus spiralis* Rosc. (Costaceae) is used in the Brazilian folk medicine as a diuretic to relieve complaints of the bladder and urethra and to expel kidney stones (Corrêa, 1984; Cruz, 1965). Pharmacological evaluation of the antiurolithic activity of the water extract of this plant in rats confirmed the folk information (Viel *et al.*, 1999). Previous phytochemical studies on *C. spiralis* have revealed the occurrence of sterols and furostanol glycosides (Willuhn and Pretzsch, 1985) and flavonol glycosides (Antunes *et al.*, 2000). As part of our program of the chemical investigation of bioactive steroidal saponins, we have now examined the rhizomes of this plant. We isolated two new steroidal saponins from *C. spiralis*, along with evaluations of their anti-inflammatory properties.

Materials and Methods

Plant material

Fresh rhizomes of *Costus spiralis* were obtained from the Ornamental Plant Garden of Federal University of Rio de Janeiro, in September 2000 and a voucher specimen is maintained in the Laboratory of Chemistry of Medicinal Plants.

General procedures

Melting points were determined by an Electrothermal 9200 micro-melting point and are uncorrected. Optical rotations were measured on a Perkin Elmer 243B polarimeter. IR spectra were measured on a Perkin Elmer 599B, negative LSIMS carried out using thioglycerol as the matrix and Cs ions accelerated at 35 kV (acceleration voltage: 8 kV). Mass spectra and GC-MS were taken on a VG Auto SpecQ spectrometer and a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer, respectively. GC was carried out with FID, using a glass capillary column (0.25 mm × 25 m, 0.25 micron, J. W. Scientific Inc.) DB-1. NMR spectra were measured in C$_5$D$_5$N (100 mg of steroidal saponin in 0.5 ml) at 25 °C with a Varian Gemini 200 NMR spectrometer, with tetramethylsilane (δ = 0.00) used as internal standard. 1H NMR spectra were recorded at 200 MHz and 13C NMR spectra at 50 MHz. Silica gel columns (230–400 mesh ASTM, Merck) and Sephadex LH-20 (Pharmacia) were used for CC. TLC was performed on silica gel plates (Kieselgel 60F$_{254}$, Merck) using the following solvent systems: (A) CHCl$_3$/MeOH/H$_2$O (65:35:10 v/v/v, lower phase) for steroidal saponins 1 and 2, (B) CHCl$_3$/MeOH (12:1 v/v) for pseudosapogenin and (C) n-BuOH/Me$_2$CO/MeOH (4:5:1 v/v/v) for monosaccharides. Spray reagents were orcinol/H$_2$SO$_4$ for steroidal saponins 1 and 2 and monosaccharides and CeSO$_4$ for pseudosapogenin.
Extraction and isolation

Fresh rhizomes (1 kg) were extracted with 80% aqueous MeOH (3 l) for 72 h at room temperature. The extract was concentrated under reduced pressure to remove most of the MeOH and the resulting aqueous phase was shaken with n-BuOH [water/n-BuOH (1:1 v/v)]. This procedure was repeated and the resulting organic phase was evaporated in vacuo, the residue dissolved in MeOH, precipitated by Et2O addition, and washed with Et2O to give a crude material (3.8 g). The residue dissolved in MeOH, precipitated by Et2O and washed with Et2O to give a crude material (3.8 g). The residue dissolved in MeOH, precipitated by Et2O addition, and washed with Et2O to give a crude material (3.8 g). Further purification by chromatography on a silica gel column provided several fractions which two TLC homogeneous compounds 1 (270 mg, Rf 0.52) and 2 (153 mg, Rf 0.55) which gave dark green colors with orcinol/H2SO4.

Compound 1

Colorless needles. [α]D25° = 106° (c 0.1, MeOH). M.p. 216–218 °C. 1H and 13C NMR data: Tables I and II.

Compound 2

Colorless needles. [α]D25° = 110° (c 0.1, MeOH). M.p. 218–220 °C. 1H and 13C NMR data: Tables I and II.

Acid hydrolysis of 1 and 2

A solution of each compound (100 mg) in 1 M HCl/1,4-dioxan (1:1 v/v; 10 ml) was heated in a sealed tube for 1 h at 100 °C. After cooling, the reaction mixture was neutralized with 3% KOH in MeOH and evaporated to dryness. The salts that deposited on addition of MeOH were filtered off and the filtrate was passed through a Sephadex LH-20 with MeOH to give the hydrolysate (93 mg) which was chromatographed on silica gel CC with CHCl3/MeOH/H2O (7:3:0.2) to yield the diosgenin (30 mg) and a sugar mixture. Identity of diosgenin was established by comparison with an authentic sample through m.p., IR, 1H and 13C NMR and EIMS. The sugar mixture was dissolved in pyridine and analyzed by silica gel-TLC in the above described solvent system. After spraying, apiose gave a weak yellow spot at Rf 0.78, rhamnose gave a green spot at Rf 0.75 and glucose gave a blue spot at Rf 0.70.

Molar carbohydrate composition and D,L configurations

The molar carbohydrate composition of 1 and 2 were determined by GC-MS analyses of their monosaccharides as their trimethylsilylated methylglycosides obtained after methanolysis (0.5 M HCl in MeOH, 24 h, 80 °C) and trimethylsilylation (Kamerling et al., 1975). The configurations of the glycosides were established by capillary GC of their trimethylsilylated (−)-2-butylglycosides (Gerwig et al., 1978).

Methylation analysis

Compounds 1 and 2 were methylated with DMSO/lithium methylsulfinyl carbamion/CH3I (Parente et al., 1985). The methyl ethers were obtained after hydrolysis (4 N TFA, 2 h, 100 °C) and analyzed as partially alditol acetates by GC-MS (Sawardeker et al., 1965).

Animals

Male BALB/c mice, weighing 15–20 g, were used. The animals were housed under standard environmental conditions and fed with standard rodent diet and water ad libitum.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated by measuring acetic acid-induced vascular permeability (Whittle, 1964). Male mice (BALB/c, 15–20 g) in groups of five were dosed orally with compounds 1 and 2 (100 µg/g body weight) and a positive control, indomethacin (10 µg/g body weight). After injection of the dye, 0.1 ml acetic acid (10 µl/g body weight) was injected intraperitoneally. Twenty minutes later, the mice were killed with an overdose of ether and the viscera were exposed after a 1 min period to allow blood to drain away from the abdominal wall. The animal was held by a flap of the abdominal wall and the viscera were irrigated with 10 ml of saline over a petri dish. The washing was filtered through glass wool and transferred to a...
Table I. Selected ¹H NMR assignments (δ (ppm), J [Hz]) of compounds 1 and 2 in C₅D₅N.

<table>
<thead>
<tr>
<th>Position</th>
<th>1</th>
<th>2</th>
<th>¹H-¹H-COSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-6</td>
<td>5.30 br s</td>
<td>5.30 br s</td>
<td>H-7</td>
</tr>
<tr>
<td>Me-18</td>
<td>0.85 s</td>
<td>0.85 s</td>
<td></td>
</tr>
<tr>
<td>Me-19</td>
<td>1.10 s</td>
<td>1.10 s</td>
<td></td>
</tr>
<tr>
<td>Me-21</td>
<td>1.22 d (6.8)</td>
<td>1.22 d (6.8)</td>
<td>H-20</td>
</tr>
<tr>
<td>Me-27</td>
<td>0.98 d (6.6)</td>
<td>0.98 d (6.6)</td>
<td>H-25</td>
</tr>
</tbody>
</table>

Aglycone characteristic proton signals

Sugar methyl group and anomeric protons

<table>
<thead>
<tr>
<th>Sugar methyl group</th>
<th>1</th>
<th>2</th>
<th></th>
<th>¹H-¹H-COSY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rha-Me</td>
<td>1.78 d (6.3)</td>
<td>1.76 d (6.3)</td>
<td>Rha-H-5</td>
<td></td>
</tr>
<tr>
<td>iGlc-H-1</td>
<td>4.98 d (7.7)</td>
<td>4.96 d (7.7)</td>
<td>iGlc-H-2</td>
<td></td>
</tr>
<tr>
<td>tGlc-H-1</td>
<td>4.82 d (7.8)</td>
<td>4.84 d (7.8)</td>
<td>tGlc-H-2</td>
<td></td>
</tr>
<tr>
<td>Rha-H-1</td>
<td>6.30 br s</td>
<td>5.80 br s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Api-H-1</td>
<td>5.95 d (3.5)</td>
<td>5.90 d (3.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results and Discussion

The fresh rhizomes of C. spiralis were extracted with methanol. After concentration under reduced pressure, the methanol extract was partitioned between water and n-butanol. Chromatographic separations of the organic phase on Sephadex LH-20 and silica gel gave compounds 1 and 2 which were detected with orcinol/H₂SO₄ reagent. Compounds 1 and 2 were obtained as colorless needles and gave positive Liebermann-Burchard test for steroidal saponins. The LSIMS showed an ion peak [M-H]⁻ at m/z 1033 which, together with ¹³C NMR spectral data (Table II), suggested the molecular formula as C₅₀H₈₂O₂₂ for compounds 1 and 2.

In addition to this, the furostanol glycosidic nature of 1 and 2 was indicated by the strong absorption bands at 3430 cm⁻¹ for the hydroxyl group and a 25R-furostan steroidal structure (813, 838 and 913 cm⁻¹, intensity 913 < 838 cm⁻¹) in the IR spectrum (Wall et al., 1952), confirmed by ¹H and ¹³C NMR spectra (Tables I and II) (Agrawal et al., 1985; Shao et al., 1997). The ¹H NMR spectral data (Table I) of compounds 1 and 2 contained a signal for the olefinic proton at δ 5.30 (br s, H-6), two secondary methyl protons at δ 1.22 (d, J = 6.8 Hz, 3H-21) and 0.98 (d, J = 6.6 Hz, 3H-27) and two angular methyl protons at δ 1.10 (s, 3H-19) and 0.85 (s, 3H-18). In addition to this, the COLOC experiments.
spectrum displayed long range couplings between H-1 of the terminal glucose and C-26 of the aglycone. The above $^1$H NMR spectral data and a comparison of the $^{13}$C NMR signals of the aglycone moiety of 1 and 2 (Table II) with those described in the literature (Agrawal et al., 1985; Shao et al., 1997) showed the structure of the aglycone to be (3β,25R)-22-hydroxyfurost-5-ene-3,22,26-triol.

In the $^{13}$C NMR spectrum of 1 and 2, a 2,4-linked inner β-D-glucopyranosyl unit, a terminal β-D-glucopyranosyl unit, a terminal α-L-rhamnopyranosyl unit and a terminal β-D-apiofuranosyl unit were clearly observed. The COLOC spectrum of compound 1 displayed long range couplings between inner glucose-H-1 at δ 4.98 and aglycone-C-3 at δ 77.9, between terminal glucose-H-1 at δ 4.82 and aglycone-C-26 at δ 74.9, between apiose-H-1 at δ 5.95 and inner glucose-C-2 at δ 77.8, and between rhamnose-H-1 at δ 6.30 and inner glucose-C-4 at δ 78.8, indicating that the apiose and rhamnose were linked to the C-2 and C-4 of the inner glucose, respectively. The COLOC spectrum of compound 2 displayed long range couplings between inner glucose-H-1 at δ 4.96 and aglycone-C-3 at δ 77.9, between terminal glucose-H-1 at δ 4.84 and aglycone-C-26 at δ 74.7, between apiose-H-1 at δ 5.90 and inner glucose-C-2 at δ 77.8, and between rhamnose-H-1 at δ 5.80 and inner glucose-C-2 at δ 77.8, indicating that the apiose and rhamnose were linked to the C-4 and C-2 of the inner glucose, respectively. The signal at δ 1.78 and 1.76 were due to the methyl group of rhamnose of 1 and 2, respectively. The methylation analyses (Parente et al., 1985) showed a terminal glucopyranose, a terminal apiofuranose, a terminal rhamnopyranose and a 2,4-linked glucopyranose for 1 and 2.

On acid hydrolysis, compounds 1 and 2 gave a pseudosapogenin, glucose, rhamnose and apiose. The pseudosapogenin was identified as diosgenin by direct comparison of TLC, m.p., IR, $^1$H and $^{13}$C NMR and EIMS with an authentic sample. The molar carbohydrate composition of 1 and 2 indicated the presence of four neutral monosaccharides: glucose/rhamnose/apiose (2.0:0.9:0.9; the molar responses of rhamnose and apiose are taken as 1.0) (Kamerling et al., 1975). Their absolute configurations were determined by GC of their trimethylsilylated (−)-2-butylglycosides (Gerwig et al., 1978). D-glucose, L-rhamnose and D-apiose were detected. Consequently, on the basis of IR, $^1$H and $^{13}$C NMR spectroscopy, LSIMS and chemical reactions, the structures of the steroidal saponins 1 and 2 were established as (3β,25R)-26-β-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl O-D-apio-β-D-furanosyl-(1→2)-O-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside and (3β,25R)-26-β-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl O-D-apio-β-D-furanosyl-(1→4)-O-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside.

![Fig. 1](image-url)

Fig. 1. (3β,25R)-26-β-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl O-D-apio-β-D-furanosyl-(1→2)-O-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside (1) and (3β,25R)-26-β-D-glucopyranosyloxy)-22-hydroxyfurost-5-en-3-yl O-D-apio-β-D-furanosyl-(1→4)-O-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranoside (2).
In order to confirm popular informations about the use of this plant against inflammatory conditions and based on literature reports of anti-inflammatory activities of steroidal saponins (La-
caille-Dubois and Wagner, 1996), this pharmacological property was evaluated for compounds 1
and 2 using the capillary permeability assay (Whittle, 1964). In the regular dose of 100 mg/kg, com-
ounds 1 and 2 showed inhibition of the increase in vascular permeability (Fig. 2) caused by acetic acid, which is a typical model of first stage inflammatory reaction. This result suggests that the ste-
roidal saponins 1 and 2 may be the potential therapeutic agents involved in inflammatory disorders justifying the use of C. spiralis in Brazilian traditional medicine.

Acknowledgements

This work was financially supported by CNPq, FAPERJ and FUJB.